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Selection of Effective Fungicide for *Colletotrichum capsici* Growth and Determination of Minimum Inhibitory Concentration of Fungicide

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The present study investigated the growth of *Colletotrichum capsici* in six different medium Potato Dextrose Agar (PDA), Oat meal Agar(OMA), Corn meal Agar(CMA), V-8 juice agar(V-8), Potato Carrot Agar(PCA) and Potato Fruit Agar(PFA). Among the different tested media, OMA supported the maximum growth significantly and PDA supported minimum growth that compared to all other media. The fungus derives food and energy from the substrate upon which they grow in nature. There is no universal substrate or artificial medium upon which all the fungi can grow and reproduce regularly. Therefore studies were conducted in different suitable media to identify surface medium for the growth and sporulation of *C. capsici*. The OMA media selected for biochemical test in *C. capsici*. The fungicides Indofil M-45 and Carbendazim were evaluated in minimum inhibitory concentration. These fungicide activity show against *C. capsici*.

Keywords: *C. capsici*, Solid Medium, Liquid Medium, Harvest of Mycelium, Antibiotic Sensitivity test, ANOVA analysis.

Colletotrichum capsici (Sydow) E. J. Butler & Bisby is an important pathogen with worldwide distribution (Than *et al*., 2008). The *C. capsici* causes anthracnose disease in the pre and post harvest condition of chilli. In India, chili cultivation is always infected by *Colletotrichum capsici* causing anthracnose (Chanchaichaovivat *et al*., 2008). Anthracnose destroys mature chili fruits during cultivation, transportation and storage causing 50% reduction of pre and post-harvest in chili fruits (Druvefors *et al*; 2004). There is losses premature fruit (50-100%), mummification of unripe green pepper fruits (Agrios., 1988). *Colletotrichum capsici* find that infects multiple hosts (Amusa *et al*., 2005). Symptoms of *C. capsici* can be observed throughout the year but they are most intense between June and September in temperate and transitional climatic zones. Concentric rings of the acervuli within the fruit spots are common symptoms. In some cases, the lesions are brown, not orange and then turn black from the formation of setae and sclerotia (Roberts *et al*., 2001).

Gopinath *et al*. (2006) found that anthracnose of chilli caused by *C. capsici* which has been become a serious problem for chilli cultivation in India. This fungus is distributed throughout the tropics and very commonly occurs in chilli growing areas. Only one way to control the *C. capsici* is by chemical substance.

MATERIALS AND METHODS

The cultures were grown under dark 12/12 hr light cycles at 27±2ºC in an incubator for the

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study growth rate of *C. capsici* in different nutrient medium. Good surface mycelium of *C. capsici* isolates grow at 27±2ºC. The growth rate of C. capsici study upon solid and liquid media.

Solid Media

The growth rate of *C. capsici* were studied on six different growth medium Potato Dextrose Agar (PDA), Oat meal Agar(OMA), Corn meal Agar(CMA), V-8 juice agar(V-8), Potato Carrot Agar(PCA) and Potato Fruit Agar(PFA). All the media were sterilized after dissolve at 15 lbs and 121°C for 15 min. *C. capsici* isolates were studies to characterize and compare isolates from three levels of sampling. To carry out the study 20 ml of each of the medium was poured in 90 mm petriplates. After solidification, 5mm diameter bits of *C. capsici* culture was placed in the centre of the petriplates with the help of sterilized cork borer. Each treatment was replicated three times. The petriplates were incubated at room temperature (27±2°C) for 10 days. Observations were taken 5 days and 10 days after inoculation of *C. capsici* culture in petriplate. The colony diameter was recorded by averaging in two directions for each plate. The data of radial growth was analyzed statistically. The composition and preparation were same for both solid and liquid media. 100 ml of the medium was added to each of 250 ml flask. Inoculums disc of 5mm size were inoculated to all flasks and incubated at 27±2°C for twelve days. Each treatment was replicated thrice. The mycelial mat was harvested and dry mycelia weight was recorded after twelve days.

Liquid Medium

The composition of liquid media (Broth) was same as in solid media except addition of agar. 50 ml of liquid media were dispensed in 150 ml conical flask and plugged with non-absorbent cotton. It was subjected, sterilization at 15 lbs and 121°C for 15 minutes in the autoclave after dissolve. Pure culture block of 5 mm diameter from 10 days old culture of *C. capsici*, cut with the help of sterilized cork borer was placed in the broth with the help of sterile forceps under aseptic conditions, replicating three times. Out of these, one replication was kept for sporulation count as per described by Palarpawar and Ghurde (1997). The rest of the replications were kept for recording dry mycelium weight of the fungus which were incubated at room temperature for a period of 10 days.

Harvest of Mycelium

After incubation period, the mycelium mats were harvested through a previously weighed filter paper (Whatman No.42). The filter papers with mycelium mats were dried in an oven at 80ºC for 24 hrs and dry weight of the mycelium was recorded.

Antibiotic Sensitivity test

This test done with two fungicides Indofil M-45 and Carbendazim on the solid media of PDA**.** The different concentration of Indofil M-45 and Carbendazim were evaluated by the disc diffusion method on the *C. capsici* culture. Agar plates were inoculated with mycelia taken from OMA media 10 days old culture. The plates were then incubated at room temperature for 24 hours. After completion of 24hrs, the plates were inverted and placed in an incubator set to 27±2.

After seven days, black spores were formed with conidia and setae on the surface of petriplates. The growth of the isolate was evaluated against Indofil M-45 (Indofil Chemicals Company, India) and Carbendazim (Insecticides India Limited, India) at different concentrations using serial dilution method (Fig. 1).

RESULTS AND DISCUSSION

The cultural studies on solid media indicated that all the six media test supported in mycelia growth of *C. capsici* isolates. *C. capsici* isolates were uniform, concentric rings, sector and irregular in solid media. The data were statistical analysis ANOVA from O.P. Sheoran Programmer, Computer Section, CCS HAU, Hisar at per medium. Among the different tested media, OMA supported the maximum growth significantly compared to all other media. Each media supported good average radial growth for *C. capsici*. Minimum but moderate radial growth was obtained from V-8(64.50mm), PFA(63.31), PCA(59.93mm) and CMA, (62.75mm) as average radial growth after addition of 5and 10 days. Pure mycelium was good growth with whitish color but dirty colored and poor mycelium was found on PDA. The growth of *C. capsici culture* maximum on (73.70mm) medium than all five medium. So OMA was significantly superior over all other media while other non significant. Kadu (1977) and Mesta (1996) obtained good growth of *C. capsici* 90 mm on potato dextrose agar. Chidanandaswamy (2001) reported that maximum

radial growth of *C. capsici* was observed on potato dextrose agar and oat meal agar followed by Richards and Sabouraud's medium.

The cultural studies of *C. capsici* on liquid media (Table 1), indicated that maximum dry mycelial weight of the fungus was obtained from V

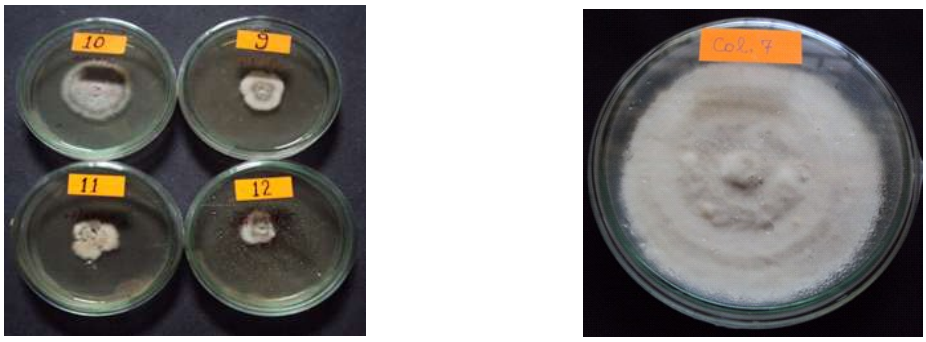

Fig. 1. The growth of *C. capsici* on PDA(Culture 9,10,11 and 12) and OMA (Culture 8) media which indicate minimum to maximum growth rate from the effect of nutrient medium

Table 1. Different type of solid media and there average sizes(in cm) after 5 and 10 days of inoculation(DAI)

S. No.	Solid Media	Error of Mean Squares	Growth Rate in Solid Media
1	PDA	25.826	Moderate growth with whitish mycelium
2	OMA	6.185	Good growth with whitish mycelium
3	CMA	2.566	Good growth with whitish mycelium
4	V-8	171.01	Poor growth with dirty white mycelium
5	PCA	0.361	Good growth with white mycelium
6	PFA	0.471	Moderate growth with white mycelium

Table 2. Mean of *C. capsici* in different solid media after 5 and 10 days of inoculation(DAI)

Isolates	Medium[1]	Medium[2]	Medium[3]	Medium[4]	Medium[5]	Medium[6]
I[1]	42.833	45.500	65.500	68.167	52.733	52.467
I [2]	56.000	56.833	81.500	83.500	70.533	72.433
I [3]	56.167	34.500	68.167	64.000	55.433	52.500
I [4]	70.667	44.167	85.667	84.667	73.133	73.033
I [5]	38.500	31.667	68.000	67.500	51.333	51.200
I [6]	55.000	46.000	84.000	60.167	69.300	73.900
I [7]	26.833	46.333	64.500	69.000	50.400	51.200
I [8]	58.667	72.500	82.000	83.500	71.067	73.667
I [9]	35.000	59.500	66.167	68.000	51.200	56.800
I [10]	43.833	77.500	76.667	84.333	72.067	78.300
I [11]	35.167	23.167	60.000	68.833	52.733	52.200
I [12]	49.833	40.000	81.500	83.500	72.833	75.567
C.D.	8.661	4.238	2.730	N/A	1.024	1.170
SE(m)	2.934	1.436	0.925	7.550	0.347	0.396
SE(d)	4.149	2.031	1.308	10.677	0.491	0.560
C.V.	10.727	5.166	2.175	17.728	0.971	1.079

Table 3. Different type of liquid media and there average mycelia weight (in mg) at 27±1^{0}C after 5 and 10 days of inoculation(DAI)

S.No.	Liquid Media	Dry Mycelia Weight (in mg)	
		5 DAI	10 DAI
1	PD broth	284.07	342.4*
2	V -8 juice broth	386.02	499.7
3	Richards's broth	343.27	465.6
Mean	386.85	337.8	435.9
SEm±	0.8943	386.85	
CD	1.768		

8 juice broth (499.7 mg) which was significantly superior over all other broth. Chidananda swamy (2001) reported good growth of *C. capsici* on Richards's broth.

Control of *C. capsici* by Indofil M-45 and Carbendazim

The *C. capsici* is only controlled by two fungicides Indofil M-45 and Carbendazim. The fungicides Indofil M-45 and Carbendazim were evaluated for minimum inhibitory concentration, activity show against the *C. capsici* (Table 1). Indofil M-45 is a board spectrum, so this fungicide popularly known as king of fungicide. It is effective against widest range of disease caused by fungal pathogen in various crops chilli. Antibiotic sensitivity test showed that diameter of the *C. capsici* colony was reduced due to Indofil M-45 and Carbendazim. Carbendazim is a systemic benzimidazole fungicide that plays a very important role in plant disease control (Quian *et al*., 1996). It was first reported in 1973 and was developed by BASF (Hicks *et al*., 1998), Hoeschst (now part of Bayer). The higher concentration gives the maximum effect which decreased with dilution. Carbendazim showed maximum efficiency even at a very low concentration of 10^{-1} 10^{-6} mg/ml as compared to Indofil at 10^{-2}mg/ml. The zone of inhibition by Indofil-45 ranges from 0.1 to 1.5 cm and 0.5 to 2.5 cm by Carbendazim. Our result satisfactory show Carbendazim inhibit the growth of *C. capsici.*

Table 4. Different concentration of pathogen on OMA media and inhibition of zonation sizes by Indofil M-45 and Carbendazim

S. No.	Concentration of Pathogen	Zone of Inhibition (cm) byIndofil M-45	Zone of Inhibition (cm) by Carbendazim
1	10^{-1}	1.5	2.5
2	10^{-2}	1.0	2.0
3	10^{-3}	0.5	1.5
4	10^{-4}	0.3	1.0
5	10^{-5}	0.1	0.5
6	10^{-6}	-	-

In conclusion, our results suggest that carbendazim can be used in lower dose for controlling *C. capsici* in chilli crop. Carbendazim showed maximum efficiency even very low concentration of 10^{-6} mg/ml as compared to that of Indofil at 10^{-2}mg/ml. So, amount of Indofil M-45 and Carbendazim use in threshold amount.

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